

Progress in the production of fungal lipases by submerged fermentation

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Abstract—Enzymatic catalysis has numerous advantages over chemical catalysts such as high specificity and high catalytic efficiency. Among enzymes, lipases are considered versatile and of vast industrial application. The main source of obtaining these enzymes are microorganisms and studies that employ filamentous fungi, in general, present promising results. Thus, it is verified the need to analyze what has been discussed in recent scientific publications in order to identify the current state of research. Therefore, this study aimed to analyze the recent literature through a bibliometric study, to identify trends and gaps in research in the area of fungal lipase production through submerged fermentation. Quantitative and qualitative analyses were used for this purpose. The analyses showed the high versatility of fungal lipases in several industrial sectors and it was possible to conclude that recent research has been focusing on the optimization of liquid cultivation media and screening by lipase-producing fungi. Moreover, there is believed to be a research gap in the investigation of the use of whole cells of fungi to reduce the costs associated with lipase-catalyzed processes and make them competitive economically.

Keywords—enzymes, filamentous fungi, biocatalysts, bibliometrics.

I. INTRODUCTION

Lipases occupy a prominent place among biocatalysts and catalyze hydrolysis reactions and long chain acyglycerol synthesis in aqueous and non-aqueous media. Its unique properties, such as selectivity and substrate specificity, position them as the most expansively used industrial enzymes (Mehta & Gupta, 2017; Sarmah et al., 2017).

Fungal lipases stand out as the main sources of lipases because of their catalytic activity, low production cost and relative ease in genetic manipulation. The production of fungal lipases has been successfully performed by submerged fermentation (FS) and solid-state fermentation (FeS) processes and depends largely on microorganism species, culture practices, medium composition and bioreactor design. In addition, immobilized fungal cells have also been used to produce lipases using various support materials (Mehta&Gupta, 2017; Geoffry&Achur, 2018 ; Salihu et al., 2012).

The versatility of enzyme-produced lipases allows their application in relevant sectors such as in the food, textile, leather, paper and detergent industry. However, as much as industrial applications of fungal lipases have increased in recent decades there is an increasing need to explore new strands, which engage both hydrolytic and synthetic reactions. The most recent applications of lipases, but already successfully

established, are seen in the synthesis of biopolymers, biodiesel production, effluent treatment, bioremediation and synthesis of drugs (Mehta & Gupta, 2017; Ray, 2015; Geoffry&Achur, 2018).

The relatively high cost of lipases combined with low operational stability remains a technological and economic bottleneck and that makes the industrial use of lipases less widespread. Research in the area suggests some alternatives such as the improvement of fermentation technologies, application of immobilization techniques and Protein Engineering (Lai et al., 2019).

As research on a given object, in a specific area of study, begins to grow there is a need to propose studies that help understand the stage of development of the object. Thus, some researchers have been looking to analyze the development of scientific research, which results in bibliometric studies. Bibliometric studies employ concepts of Bibliometrics, a statistical method used to evaluate and quantify the number and growth trend of a given subject (Mao et al., 2018). This analysis allows the visualization of the scientific and technological advances already made (Moreira; Xavier; Lira, 2018). Thus, it is believed that it is possible that technological advances in the area of research on fungal lipase production via FS can be identified through bibliometric studies.

The objective of this work is to contribute to the knowledge about trends and research gaps on fungal lipase

production by submerged fermentation observed in recent literature. For this purpose, quantitative and qualitative analyses of recent publications were performed (published between 2010 and 2019), through a bibliometric study.

II. METHODOLOGY

The bibliometric study was conducted in two stages. The first stage, the quantitative analysis, was performed in order to obtain the quantitative data of the publications and present them in the form of graphs. The second stage, the qualitative analysis, aimed to describe the themes discussed in the analyzed studies and discuss possible implications relevant to the objective of this work.

The database employed in the research was the Web of Science platform (WoS), belonging to Clarivate Analytics. This basis was chosen because it was considered a data source with broad indexation of journals of scientific importance and because it has a great diversity of indexed metadata, which include the authors of publications, their affiliations and countries and basic bibliographic information (Paul-Hus; Desrochers; Costas, 2016; Olensky; Schmidt; Van Eck, 2016; Pereira et al., 2019).

Initially, an exploratory research was conducted, searching the WoS database for the term "lipases" and selecting the mode of search for topics. The period considered included the years 1945 (year limit of registration of works in the database) to 2019 and only papers published in English, Portuguese and Spanish (Margon; Freitas; Pinotti, 2018; Yaoyang&Boeing, 2013).

To refine the results obtained in exploratory research, the date of publication of the papers was restricted from 2010 to 2019, in order to identify research trends on recurrent lipases in the last decade. The results were filtered by the type of study, considering as relevant results only original articles, reviews and protocols of procedures.

The results of the research, carried out according to the methodology described above, were again refined with the insertion of new terms ("submerged" and "fungi" or "fungal"). In this step, a citation report was created with WoS data analysis tool. Based on the data obtained, graphs were constructed that presented the temporal evolution of the number of publications according to the years and countries related to the research (Catelan&Pinotti, 2019).

The articles found were read and selected according to content adequacy to the objective of this study. The articles, listed in Table 1, were discussed for their content. From the discussion, a table was elaborated (Table 2), which presents, succinctly, the main operational conditions of fungal lipase production by submerged fermentation studied by the studies analyzed here.

Based on the articles selected in the refined research, a SWOT analysis (Table 3) was performed, which is a tool that detects and corrects apparent problems in each area and allowed a better understanding of the proposed analyses. SWOT analysis consists of ways to analyze strengths, weaknesses, opportunities and threats and ways to convert threats into opportunities and weaknesses into forces (Margon; Freitas; Pinotti, 2018; Souza et al., 2018).

III. RESULTS AND DISCUSSION

Exploratory research in the Web of Science database, using only the term "lipases" and from 1945 to 2019, found 9,082 results. From the data obtained, a graph was elaborated that presents the number of publications according to the years in the period considered (Figure 1).

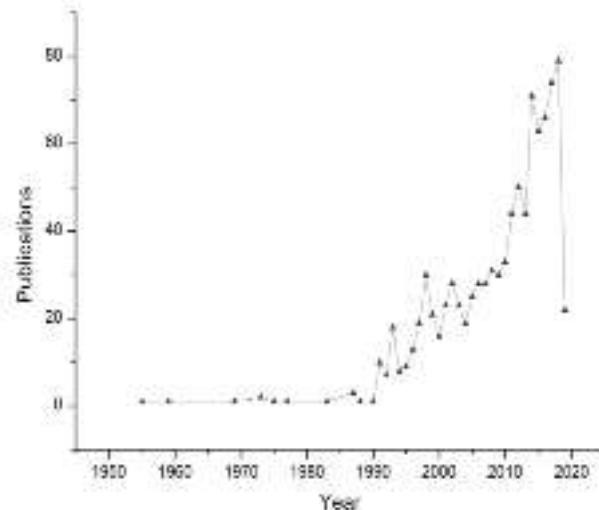


Fig. 1: Results of the research term "lipases" by the number of publications in the period 1945-2019

Source: Adapted from Web of Science by authors (2019)

Figure 1 shows that the first publications related to lipase research occurred in 1950. Although the scientific community's interest in lipase study apparently began in 1950, research began to intensify only in 1990, when there was a significant expansion in the number of scientific publications on the subject, noted by a 419% increase in the number of publications between 1990 and 1991.

In order to refine the results obtained previously, the time space was reduced for the period between 2010 and 2019 and new research terms were added, aiming to select only recently published papers, such as shown in Figure 2.

Figure 2 shows that in the period considered only in 2013 there were no publications related to the limited research clipping in this study. In 2011 and 2014, there was a slight increase in the number of published papers, with 4 publications published in 2011 and 5 publications in 2014.

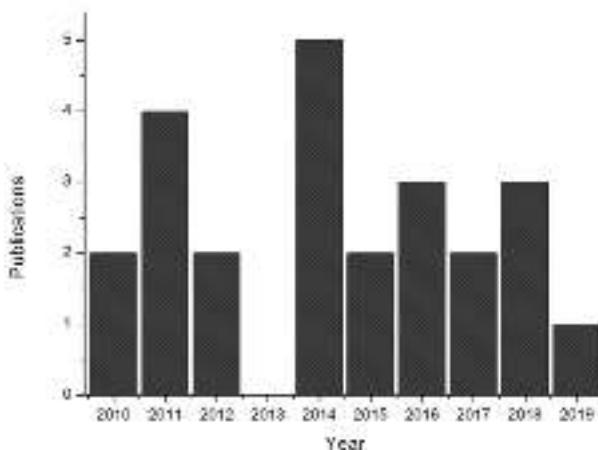


Fig. 2: Publications of studies per year in the temporal space understood from 2010 to 2019

Source: Adapted from Web of Science by authors(2019)

In parallel, the analysis of the number of annual publications was also listed the most prominent countries in the number of publications, between the years 2010 to 2019, as shown in Figure 3.

Figure 3 shows that the country with the highest number of publications is Brazil, with a total of 10 published papers. Secondly, India is in five polls, followed by Pakistan with 3 publications, and finally Germany with 2 publications. The other countries listed in Figure 3 presented only 1 publication each in the period considered.

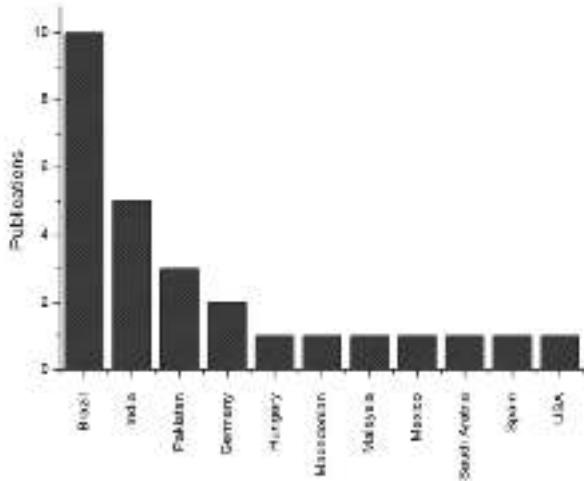


Fig. 3: Publications by countries in the temporal space understood from 2010 to 2019

Source: Adapted from Web of Science by authors (2019).

As we can see in Figures 2 and 3, 23 articles were obtained using the time criterion (2010 to 2019) and the words ("lipases", "submerged fermentation" and "fungal" or "fungi"). The complete reading of scientific publications was carried out and careful analysis was carried out about the adequacy of its content to the scope of this work, in

order to select only studies considered relevant, that is, only studies that addressed research related to the production of fungal lipases produced by submerged fermentation. Table 1 shows the selected papers as well as their respective authors, publication date and total number of citations and Table 2 presents the operating conditions found in this research.

Table 1: Titles, authors and total number of citations of selected studies after selection and complete reading of the studies

Title	Authors	Total number of Citations
Lipase from marine Aspergillus awamori BTMFW032: Production, partial purification and application in oil effluent treatment	Basheer et al. (2011)	37
Surface response methodology for the optimization of Lipase production under Submerged Fermentation by filamentous fungi	Colla et al. (2016)	17
Production and Characterization of Lipases by Two New Isolates of Aspergillus through Solid-State and Submerged Fermentation	Colla et al. (2015)	16
Production and characterization of Lipases and immobilization of whole cell of the thermophilic Thermomucor indicus eudatiae N31 for transesterification reaction	Ferrarezi et al. (2014)	14
Evaluation of lipase production using different strains of microorganisms isolated from dairy effluent through submerged fermentation	Roveda et al. (2010)	8
Selection of Lipase-Producing Microorganisms through Submerged Fermentation	Colla et al. (2010)	4
Coconut oil induced production of a surfactant-compatible lipase from Aspergillus tamarii under submerged fermentation	Das et al. (2017)	2
Mutagenic strain improvement of Aspergillus niger (MBL-1511) and optimization of cultural conditions for boosted lipolytic potential through submerged fermentation	Sidra et al. (2016)	2
Extracellular Lipase Production by Aspergillus nidulans (MBL-S-6) under Submerged Fermentation	Niaz et al. (2014)	2

Source: Adapted from Web of Science by authors (2019)

Basheer et al. (2011) reported the potential of oily effluent bioremediation by extracellular fungal lipases synthesized by a marine isolate, identified as *Aspergillus awamori*. The production of lipases by *A. awamori* was influenced by the following factors: temperature, incubation time, soybean meal concentration and concentration of KH_2PO_4 and $(\text{NH}_4)_2\text{SO}_4$.

Colla et al. (2016) evaluated the influence of nine distinct factors (carbon source, nitrogen source, nitrogen concentration, inducer, concentration of inducers, fungus species, pH and agitation) on production by submerged fermentation of lipases. Fungi *Aspergillus niger* and *Aspergillus flavus* were selected as good producers and among the factors studied only the concentration of nitrogen source (yeast extract) and pH were considered as statistically significant factors.

Colla et al. (2015) they studied the production of extracellular lipases by the genus *Aspergillus* by FS and concluded that the enzymes produced by submerged fermentation were more thermally stable than the lipases obtained by solid state fermentation and presented high stability in acid pH (3.5-6.5).

Ferrareziet al. (2014) concluded that the catalytic application of whole cells of the filamentous fungus *Thermomucor indicae-eudatiae N31* immobilized in vegetable sponges indicated can be considered promising in synthesis reactions, since esterification and transesterification yields were considered satisfactory (28% and 13.2% using oleic acid and soybean oil, respectively), with a view to the possibility of employing the unpurified enzyme in several processes. The reactions mentioned above are essential to produce biodiesel via enzymatic catalysis. In this context, immobilized intact cells present the possibility of reducing the overall costs of biocatalyst lipase processes due to the possibility of reuse of enzymes and the use of unpurified lipase.

Roveda et al. (2010) aimed to evaluate the production of lipases by fungi isolated from effluent from milk production. Fungal isolates from the genus *Penicillium*, *Aspergillus*, *Trichoderma* and *Fusarium* were obtained. In submerged fermentation, fungi E-9 (*Aspergillus*), E-21 (*Aspergillus*) and E-20 (*Penicillium*) were the ones that presented the highest enzymatic activities, using as a means of cultivation the effluent collected from the treatment system. This study indicates that fungal strains isolated from effluents have the potential to be used in the treatment of fatty effluents, as well as indicate that the use of effluents as means of cultivation can be feasible to reduce the production costs of lipases.

Colla et al. (2010) they are screening lipolytic fungi by submerged fermentation. Fungal strains were isolated from milk and soil samples contaminated with diesel oil. The largest producers were identified as the fungi *Penicillium E-3* with maximum lipolytic activity of 2.81 U, *Trichoderma E-19* and *Aspergillus O-8* with maximum activities of 2.34 and 2.30 U, respectively.

Das et al. (2017) found that the maximum production of lipases by the fungus identified as *Aspergillus tamirii* was obtained by optimizing operating and nutritional conditions, in liquid medium supplemented with mineral salts and coconut oil, as an inducer of the production of lipolytic enzymes. The authors reported that the addition of crude lipases in detergent improved by approximately 2.2 times the ability to remove oily stains and suggested their application in the formulation of detergents.

Sidra et al. (2016) showed that there was a significant increase (147.27%) in extracellular lipase activity by a mutant produced from *Aspergillus niger* (MBL-1511) in relation to the wild lineage of that microorganism. Optimal experimental conditions for submerged lipase production such as inoculum concentration, temperature, fermentation time, pH, carbon sources and inducer were studied, achieving an improvement of approximately 30% in relation to the maximum lipolytic activity presented by the mutant produced.

Niazet al. (2014) identified a coded microorganism such as *Aspergillus nidulans* MBL-S-6 and conducted a study to determine optimal conditions to produce extracellular lipases via FS. A gradual increase in enzymatic production and specific activity was observed when experiments included the presence of rice meal, increased fermentation time, variations in inoculum size and increased fermentation medium volume.

The analysis of the results of the research conducted indicates that research efforts in the production of fungal lipases by FS are concentrated in the screening of fungal strains, lipid-producing skills, and on the optimization of the cultivation medium and operational conditions, which is in accordance with the conclusions found by Singh & Mukhopadhyay (2012, p. 513). In addition, the use of vegetable oils from different sources, such as inducers of lipase production was observed in virtually all studies (see Table 2), indicating the strong dependence of a lipid inducer to provide lipase production.

Moreover, the versatility of industrial application of fungal lipases is evident, since its potentiality was pointed out for use in varied applications such as biosurfactants formulation, in the treatment of oily or greasy effluents

and in the biofuels industry, as well as scored in the studies by Joshie&Kuila (2018, p.243), Geoffry&Achur (2018, p.250) and Singh & Mukhopadhyay (2012, p.513).

The reduction in production costs proved to be a recurring topic in the research conducted, since the high cost of lipases is an obstacle to their large-scale commercial employment. According to Kumar, Sharma & Kanwar (2012), "future developments in low-cost production and purification technologies would reduce the cost of these enzymes and allow increased commercial applications." In this sense, the use of agro-industrial waste as unique sources of carbon and effluents rich in organic matter as means of cultivation are established as viable alternatives, the reuse of immobilized lipases and also the use of intact cells, which do not require complex purification processes, as highlighted by Salihu et al.(2012), Shelatkarm&Padalia (2016) and Wachtmeister (2016). The presence of few studies in the sample of studies analyzed (1 study in 10 results) on the use of whole cells of fungi as biocatalysts indicates a possible gap in the clipping of the research area considered here, which is believed to be a potential area of research for the future.

The advantages of the use of lipases as industrial biocatalysts are widely explored in the analyzed work. Simultaneously, some disadvantages were also listed, with long reaction times and low yields mentioned more frequently, demonstrating that these are the main challenges to be faced in this context.

The strengths, weaknesses, threats and opportunities found after the analysis of the search results were listed in the SWOT matrix (Table 3).

Table 3: SWOT analysis of the sample of selected articles

Forces	Weaknesses
-High catalytic efficiency, high selectivity, great versatility and environmental advantages.	-High cost of lipases makes bio catalyzed processes impossible on a large scale.
Threats	Opportunities
-Low reaction yields and long reaction times are verified in lipase-catalyzed reactions.	-Application of immobilized cells in biofuel catalysis reactions and effluent treatment;

Source: Prepared by the authors (2019)

IV. CONCLUSION

Based on the quantitative and qualitative analyses carried out from recent publications it was possible to satisfactorily identify the main topics treated, trends and possible gaps in research on fungal lipase production by submerged fermentation. In general, lipases are versatile

enzymes that have numerous industrial applications. Regarding the production of lipases, it was observed that microbial lipases are essential sources for obtaining these enzymes and studies that used filamentous fungi are very promising. The scope of the analyses performed allows us to observe that the interest of the scientific community is concentrated in the optimization of liquid cultivation media and screening by lipase-producing fungi, identified as trends for the future of research in the production of fungal lipases via submerged fermentation. In addition, it is believed that there is a gap in research on the use of intact cells of fungi, as a way of reducing costs by eliminating purification steps of the enzymes produced, which has the potential to make the processes bio catalyzed by lipases more competitive.

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Conditions/ Authors	Niaz et al. (2014)	Sidra et al. (2016)	Das et al. (2017)	Colla et al. (2010)	Roveda et al (2010)	Ferrarezi et al. (2014)	Colla et al. (2015)	Colla et al (2016)	Basheer et al. (2011)
F	Aspergillus nidulans	Aspergillus niger	Aspergillus tamarii	Penicillium E-3	Aspergillus and Penicillium	Thermomucor indicaesudaticaen31	Aspergillus.	Aspergillus flavus	Aspergillus awamori
I	---	Sunflower oil	Coconut oil	Olive oil	Olive oil	Soybean oil	Soybean oil	Soybean oil	Rice bran oil
Tl	Extracellular	Extracellular	Extracellular	Extracellular	Extracellular	Immobilized whole cells	Extracellular	Extracellular	Extracellular
T and pH	40°C and 7.0	30°C and 6.0	25°C and 6.0	30°C and 6.0	30°C and 6.5	45°C and 6.0	30°C and 7.0	30°C and 5.0	35°C and 3.0
A	200 rpm	200 rpm	120 rpm	---	130 rpm	150 rpm	160 rpm	160 rpm	150 rpm
Tf	3 days	4 days	7 days	5 days	7 days	10 days	4 days	10 days	5 days
Al	33.33 U.mL ⁻¹	22, 0 U.mL ⁻¹	25.83 U.mL ⁻¹	2.81 U	2.25 U	108 U.g ⁻¹	4.30 U.mL ⁻¹	---	495, 0 U.mL ⁻¹

Fig. 2: Summary of the operating conditions studied in the production of lipases by FS found in the selected sample of articles, in which: F=Best lipase-producing fungi, I=Inducer, Tl=Type of lipase, T and pH=crop temperature and optimum pH, A=agitation, Tf=fermentation time, AL=Maximum Lipolytic Activity found

Source: Prepared by the authors (2019)